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TITLE: A Novel Approach to Monitoring Prostate Tumor Oxygenation: Proton MRI of the Reporter Molecule Hexamethyldisiloxane

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13. ABSTRACT (Maximum 200 Words)

Growing evidence from experimental and clinical studies confirms that solid human tumors have foci of hypoxic cells, which have a profound influence on the therapeutic outcome of cancer chemotherapy and radiotherapy. A strong argument therefore exists for assessing the hypoxic fraction of tumors prior to patient treatment, and to tailor this treatment accordingly. It has been shown that there is linear relationship between R1 of hexamethyldisiloxane (HMDSO) and pO2, and the R1 of HMDSO is insensitive to various ions and minimally sensitive to temperature. The primary sequence for in vivo T1 measurement with water and fat suppression has been established, which can successfully monitor the global tumor oxygenation responding to respiratory intervention by measuring HMDSO spin-lattice relaxation time using spectroscopy. Although the imaging technique still needs to be optimized in tumor, pO2 maps accompanying respiratory intervention has been obtained in rat muscle. So, Hexamethyldisiloxane shows promise as a reporter molecule to measure tumor oxygenation by 1H MRS and potentially by MRI. This opens new opportunities for MR tumor oximetry, particularly since HMDSO is used widely in biomedical materials and as an ingredient in consumer products; and HMDSO is reported to have minimal toxicity.

14. SUBJECT TERMS
Hexamethyldisiloxane, hypoxia, Prostate tumor, Proton MRI, Oximetry

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Introduction:

Growing evidence from experimental and clinical studies confirms that solid human tumors have foci of hypoxic cells, which have a profound influence on the therapeutic outcome of cancer chemotherapy and radiotherapy: the level and severity of hypoxia can be a strong prognostic factor of disease progression and survival. A strong argument therefore exists for assessing the hypoxic fraction of tumors prior to patient treatment, and to tailor this treatment accordingly.

Baseline pO_2 and dynamic changes with respiratory intervention has been measured extensively in rat prostate tumors by ^{19}F MRI (*FREDOM*, *F*luorocarbon *R*elaxometry using *E*cho planar imaging for *D*ynamic *O*xygen *M*apping) based on the spin-lattice relaxation of Hexafluorobenzene (HFB), which provides spatially resolved maps of tumor oxygenation at depth and allows monitoring of dynamic changes at specific locations. However, for clinical application ^{19}F NMR is not yet widely available. A proton MR analog of HFB could facilitate immediate widespread oximetry. We have identified hexamethyldisiloxane (HMDSO) as a potential reporter. HMDSO has extensive symmetry and a single proton resonance well removed from water.

We had three specific aims for this project:

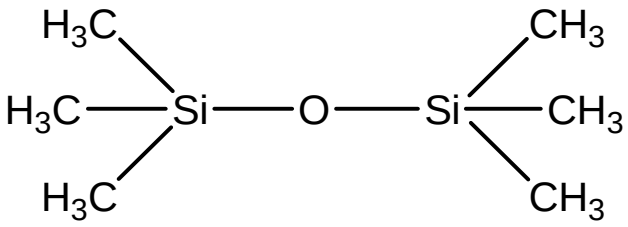
Task1. Characterize the effects of external factors on T1(spin-lattice relaxation time) of HMDSO in vitro and get the calibration curves, Month 1-6

Task2. Develop relevant MR pulse sequences with H_2O suppression for imaging. Establish experimental prostate tumor models and measure the retention time of HMDSO in tumors, Month 6-12

Task3. Make pO_2 maps and measure prostate tumor oxygen dynamics with respect to growth rate and respiratory challenge, Month 12-24

Body:

Hexamethyldisiloxane (HMDSO) is used widely in biomedical materials and as an ingredient in consumer products, such as a thin polymeric coating on suture for cardiovascular surgery, or the thin layer onto the inner surface of plasma-modified small diameter tubing, etc. HMDSO is reported to have minimal toxicity. A previous test, in vitro indicated that the spin-lattice relaxation time of HMDSO was sensitive to oxygen tension. HMDSO is highly hydrophobic, and therefore can be injected intratumorally at specific sites and will not diffuse in the tumor. Moreover, the boiling point of HMDSO is 99-100^{o}C. In addition, HMDSO has only one ^{1}H signal and the chemical shift difference between HMDSO and H_2O is about 4.7ppm. Thus, we believe HMDSO may be appropriate for measuring tumor pO_2 by ^{1}H MRS and MRI. In this proposal, I proposed to develop proton MR techniques using this new reporter molecule, HMDSO, to assess prostate tumor oxygenation in different prostate tumor sublines with several selected levels of histological differentiation.



My statement of work was:

Task1. Characterize the effects of external factors on T1(spin-lattice relaxation time) of HMDSO in vitro and get the calibration curves, Month 1-6

Measure the influence of

a. different temperatures on T1 of HMDSO
b. different metal ions on T1 of HMDSO
c. different B_0 on T1 of HMDSO
d. different pO_2 on T1 of HMDSO at various temperature, metal ions and B_0.

Task2. Develop relevant MR pulse sequences with H_2O suppression for imaging. Establish experimental prostate tumor models and measure the retention time of HMDSO in tumors, Month 6-12

a. Become proficient with state of the art NMR e.g. echo planar imaging
b. Design and implement special pulse sequences to suppress the resonance of H_2O while meaning relaxation
c. Become proficient with surgically creating tumor pedicles and implantation of prostate tumors
d. Use three selected sublines of the Dunning prostate tumor in rats, allow to grow to 1 cm diameter and measure the retention time of HMDSO

Task 3. Make pO_2 maps and measure prostate tumor oxygen dynamics with respect to growth rate and respiratory challenge, Month 12-24

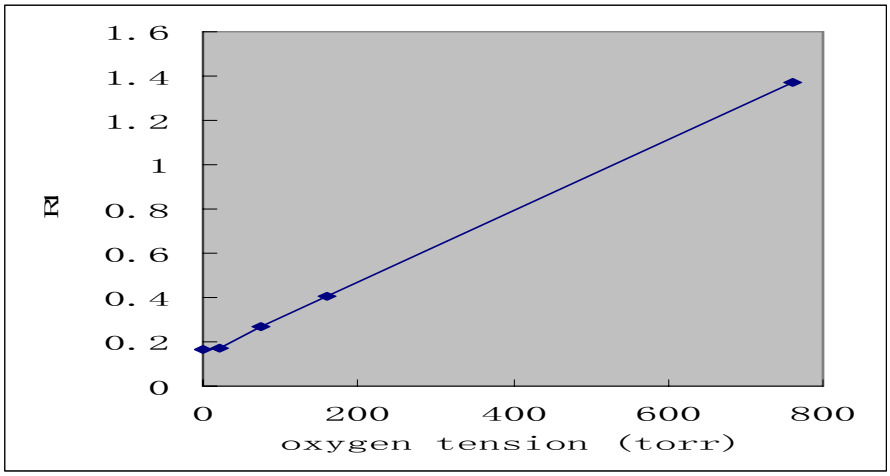
a. Measure baseline pO_2 of tumors and make the pO_2 map
b. Measure tumor oxygen dynamics with respiratory intervention
c. Excise and process tumor tissue for histology
d. Prepare manuscripts and reports (month 18-24)

Key research accomplishments:

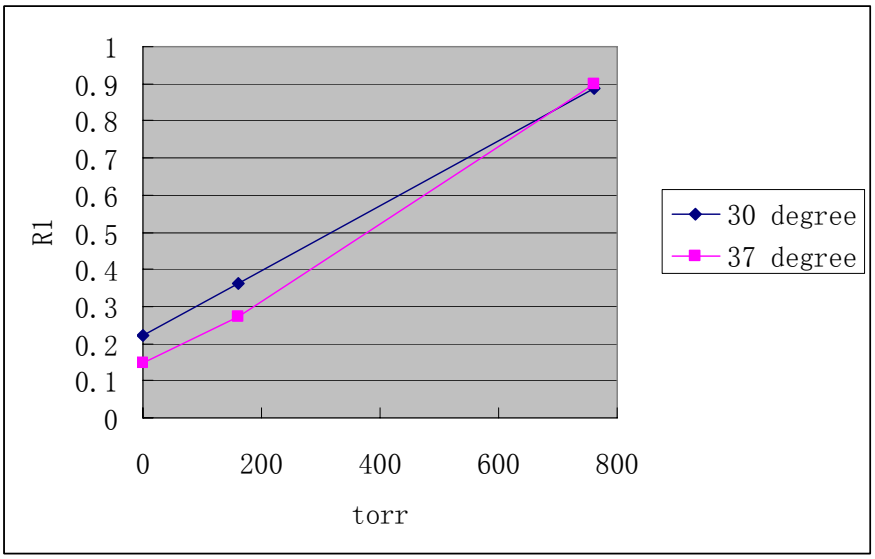
Task1. In order to characterize the effects of external factors on T1(spin-lattice relaxation time) of HMDSO in vitro and get the calibration curves, we measured the influence of: a). different ions on T1 of HMDSO, b). different temperatures on T1 of HMDSO, c) different B0 on T1 measurement, d) different pO2 on T1 measurement

1. T1 of HMDSO with different oxygen tension was measured on Varian 200 MHz and 600 MHz.

200MHz:

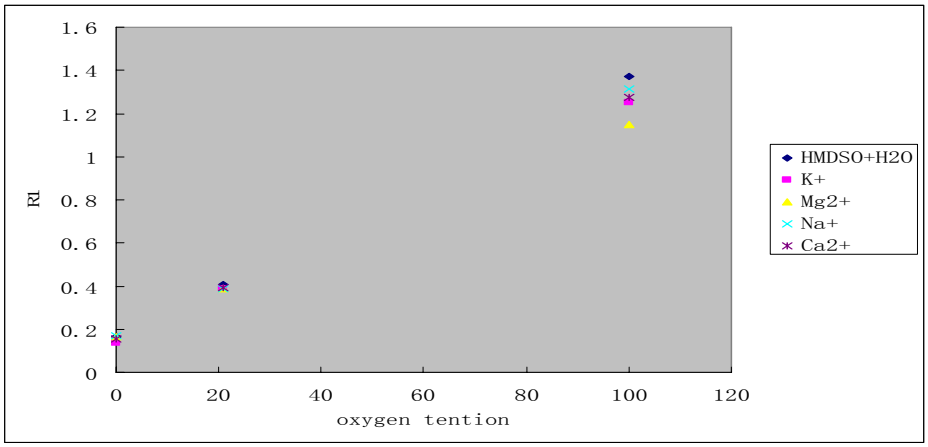


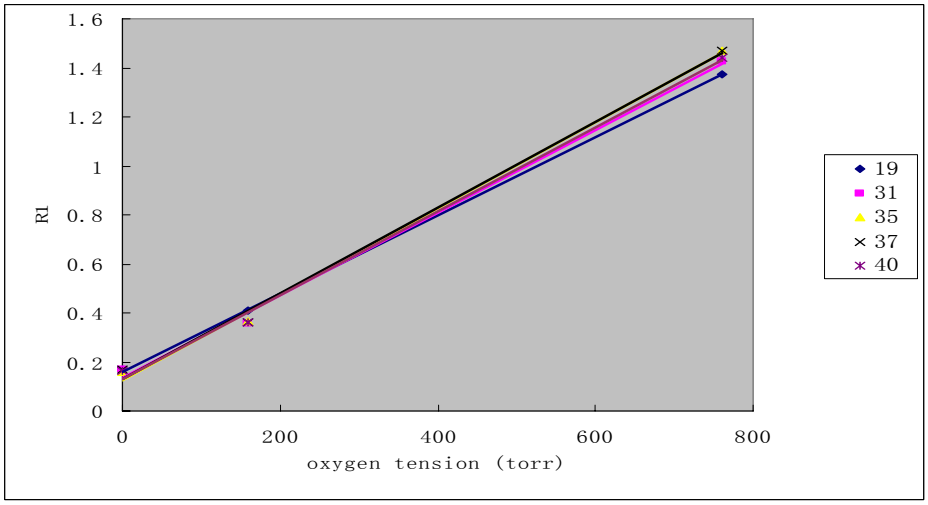
600 MHz



From above data, we can conclude that there is linear relationship between oxygen tension and spin-lattice relaxation rate. The correlation equation at 200 MHz is: R1=0.15+0.00168x (X is oxygen tension (torr) at room temperature. The correlation equation at 600MHz is: R1=0.22+0.0012X at 30 centigrade.

2. Prepare the solution of Na^+, K^+, Mg^{2+}, Ca^{2+}, the mixture of HMDSO+H2O, and the ion concentration is calculated according to the proportion of various ion in vivo. The T1 of these solutions were measured on 200MHz with various temperature and oxygen tension. It was found that there is no significant influence of various ions on spin-lattice relaxation rate, especially in the condition of lower oxygen tension that tumors have. R1 of HMDSO is only minimally sensitive to temperature. The correlation equation at 37°C is R1=0.13+0.00175x (X is oxygen tension (torr)).





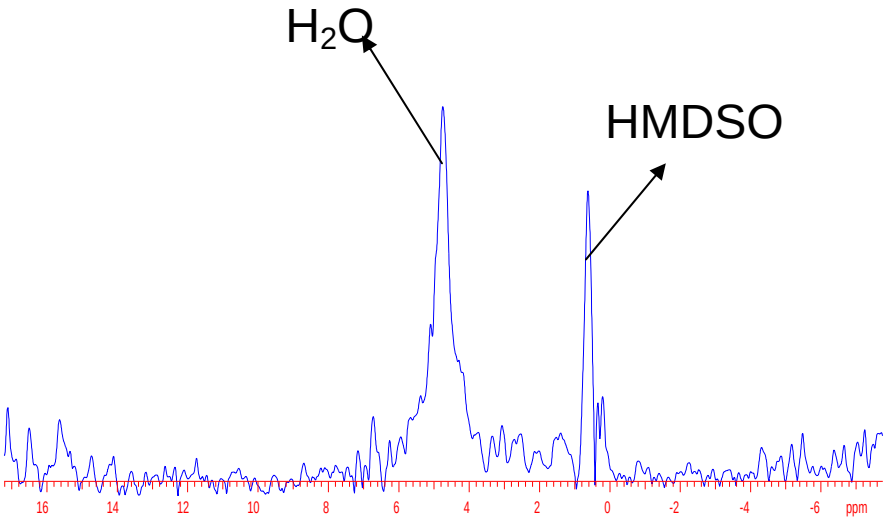
Task2. Develop relevant MR pulse sequences with H_2O suppression for imaging. Establish experimental prostate tumor models and measure the retention time of HMDSO in tumors

a. I became proficient with surgically creating tumor pedicles and implantation of prostate tumors

For thigh tumor: a flap of depilated skin is raised from the thigh of young adult male Copenhagen rat and held in position. A 1cm incision is made. A piece of fresh tumor tissue ($2x2x2mm^3$) is implanted under the skin and the cut closed with a wound clip.

For pedicle tumor: A flap of depilated skin is raised from the body of young adult male Copenhagen rat and held in position with a non-traumatic curved bull-dog clip. A 3cm incision is made through the skin using the curved edge of the clip as guide. Would clips are used to join the edge of the skin producing a tube resembling a suitcase handle. Two weeks later, the clips are removed and the distal end of the pedicle severed. A piece of fresh tumor tissue ($2x2x2mm^3$) is implanted in the lumen and the cut closed with a wound clip.

b. I used selected sublines of the Dunning prostate tumor in rats to measure the retention time of HMDSO in tumor. 100 μl HMDSO was injected intratumorally into Dunning prostate 3327 AT1 and H tumor, and PRESS pulse sequence with water suppression was used to acquire data. It was shown that HMDSO can remain at least 24hr within prostate tumor.



c. With the assistance of Dr. Vikram Kodibagkar, the MR scientist, a special pulse sequence to suppress the resonance of H2O and fat while meaning relaxation is designed and implemented.

For T1 measurements by spectroscopy, a pulse burst saturation recovery (PBSR) sequence consisting of 20 nonselective 90^o pulses for saturation of signal followed by a delay tau for magnetization recovery and a single, frequency selective 90^o pulse for signal detection was used. T1 values were measured using this sequence with the ARDVARC (Alternating Relaxation Delays with Variable Acquisitions for Reduction of Clearance effects) protocol. For imaging experiments a spin-echo EPI based PBSR pulse sequence is being designed for measuring T1 values. The sequence consists of a) a train of 20 non-selective 90^o pulses for saturation of signal followed by a delay tau for magnetization recovery, b) three optional CHESS pulses for frequency selective saturation of residual water and fat immediately followed by c) spin-echo EPI detection with a slice selective 90^o pulse and a frequency selective 180^o pulse.

Task 3a. Measure baseline pO_2 of tumors and make the pO_2 map (Dr. Vikram Kodibagkar, a MR scientist in the Department of Radiology at UTSW collaborated and assisted me in the task)

We reported that the pulse sequence (Fig1) with fat and water suppression developed by Dr. Kodibagkar worked very well with phantom. When it was used in Copenhagen rat thigh, the water and fat suppression are very effective, and baseline pO_2 map was obtained (Fig 2). However, when this pulse sequence was used in tumor, water and fat suppression was incomplete; therefore, HMDSO imaging was less satisfactory. This maybe can be ascribed to different physiological characteristics of tumor and muscle, and tumor has more fat.

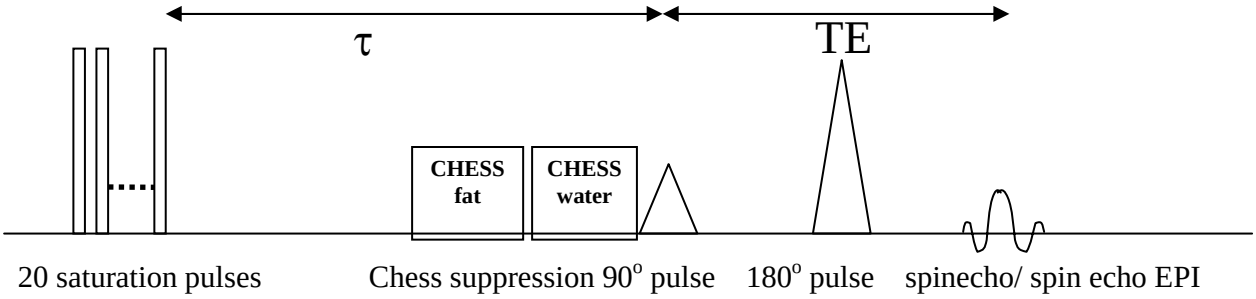


Fig 1. Pulse sequence for HMDSO relaxometry with fat and water CHESS suppression. For spectroscopy: both π/2 and π pulses are frequency selective for the HMDSO resonance. For imaging: a frequency selective π/2 and a slice selective π pulse with EPI detection are used.

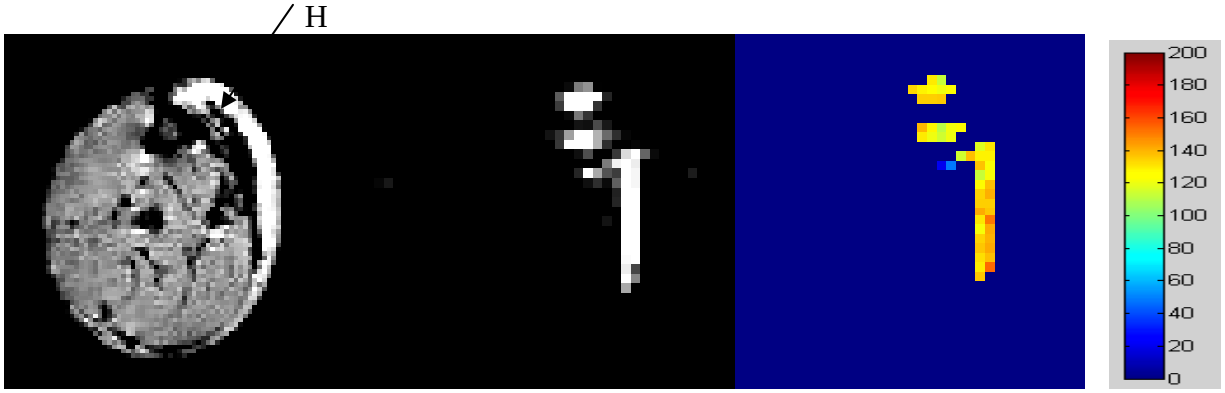


Fig 2. (a) T2 weighted spin-echo image of rat thigh showing hyperintense HMDSO (H) (b) comparative proton density weighted EPI image with fat and water suppression. (c) Corresponding pO_2 map showing a mean $pO_2 = 125 \pm 20$ torr

Task 3b. Measure tumor oxygen dynamics with respiratory intervention

I. As a proof or principle, the global oxygen dynamics curve (Fig 3) and pO_2 maps (Fig4) responding to respiratory challenge is successfully measured using pulse sequence in rat thigh muscle. During the experiment, the rat was breathing air- oxygen- air.

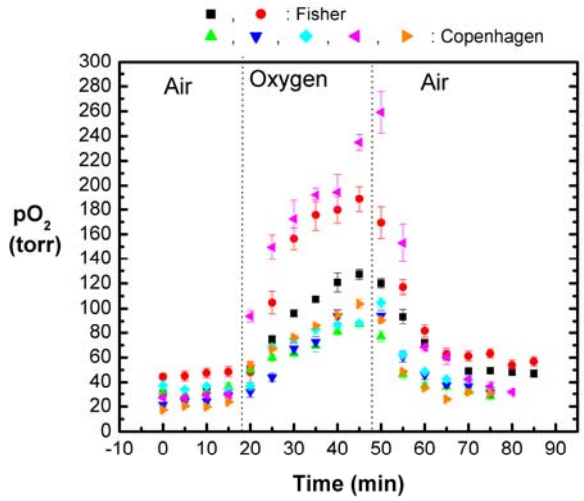
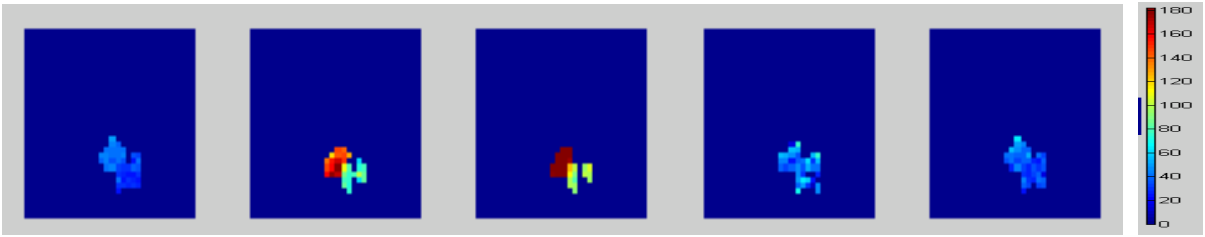


Fig3. Rat thigh muscle oxygen dynamics with respect to respiratory intervention (7 individual investigations in 2 rat strains)



Air(35±7torr) 15 min O2(77±8) 30 min O2(134±43) 15 min air(44±15) 30 min air(37±9)

Fig 4. Rat thigh muscle pO_2 map with respect to respiratory intervention

II: We measured the global tumor oxygen dynamics using frequency selective spectroscopy for the HMDSO resonance (Fig1). 120 μl HMDSO was injected intratumorly using 32 gauge Hamilton needle to distribute in the peripheral region of the AT1 tumor. MRI studies were performed on a Varian 4.7 T system. During the experiment, the rat breathed air (15min)- oxygen (30min)- air (20min) (tumor 62R2L2 is exceptional, 10min air-25min oxygen- 15min air). Fig 5 showed the pO_2 value for each tumor with respect to the breathing sequence. Table 1 and Fig 6 showed average pO_2 values for air-oxygen-air.

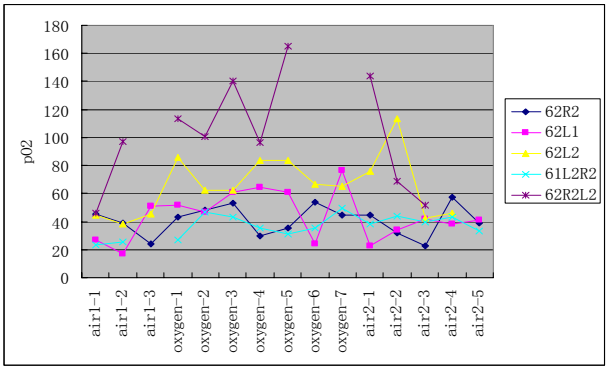


Fig5. Tumor oxygen dynamics with respect to respiratory intervention for rat prostate AT1 tumor

Table1. Global tumor oxygen dynamics with respiratory intervention

Tumor size(cm3)		Air	oxygen	air
1.5x1.5x1.0	**R1**	0. 1825	0. 1963	0. 1879
	pO_2	36	44	39
	SD	10. 9	8. 8	13. 0
1.4X1.7X2.0	**R1**	0. 175	0. 2146	0. 1814
	pO_2	32	55	35
	SD	17. 5	16. 7	7. 7
1.5x1.3x1.0	**R1**	0. 194	0. 2459	0. 2255
	pO_2	43	73	61
	SD	3. 7	10. 8	34. 2
1.6x1.4x1.3	**R1**	0. 1624	0. 1865	0. 1889
	pO_2	25	38	40
	SD	1. 2	8. 4	4. 2
1.4x1.2x0.8	**R1**	0. 2436	0. 3566	0. 271
	pO_2	71	123	87
	SD	36	29	49

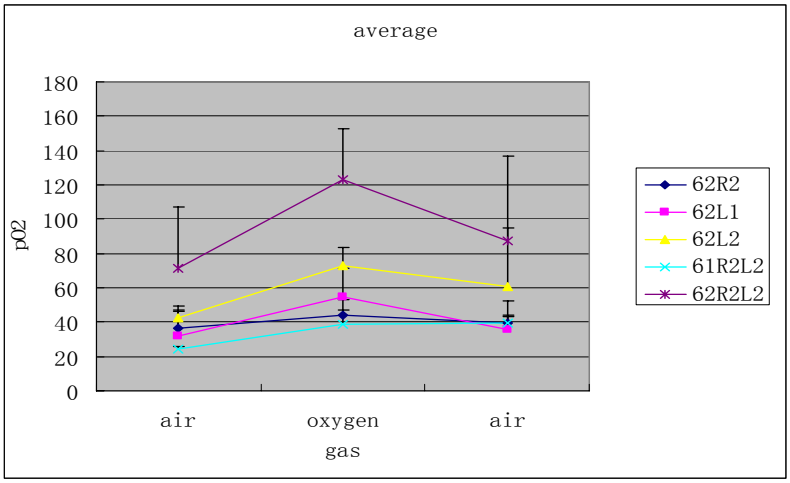


Fig6. The average pO_2 value during respiratory intervention

III. Oximetry based on HMDSO imaging in tumor is still under development. It is more difficult to implement than in muscle. While continuing to tackle this problems, I have explored application of BOLD (Blood Oxygen Level Dependent) imaging in Dunning prostate rat tumor. During the experiment, the rat was breathing 15min air-25min oxygen- 20min air. The data were normalized using the initial intensity. Fig 7 shows the signal intensity changed with gas challenge for individual data point. Table 2 and Fig 8 shows average values for air-oxygen-air.

Table 2. Normalized BOLD imaging intensity with respiratory intervention

Tumor number	**60R1**	**61R2**	**60R2L2**	**60R1L1**
Tumor size (cm3)	1.7x1.8x1.6	2.4x2.2x1.8	2.1x1.9x1.6	2.3x2.1x1.9
Respiratory intervention	The normalized signal intensity average			
Air	0.9915	0.9988	0.997	0.9921
Oxygen	0.992	1.0164	1.002	1.0516
Air	1.0242	0.9837	0.9949	1.0637

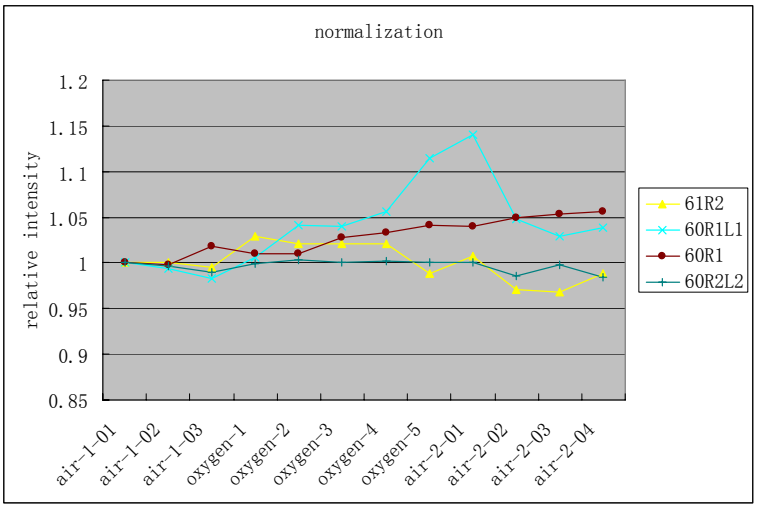


Fig 7. BOLD imaging signal intensity for AT1 tumor

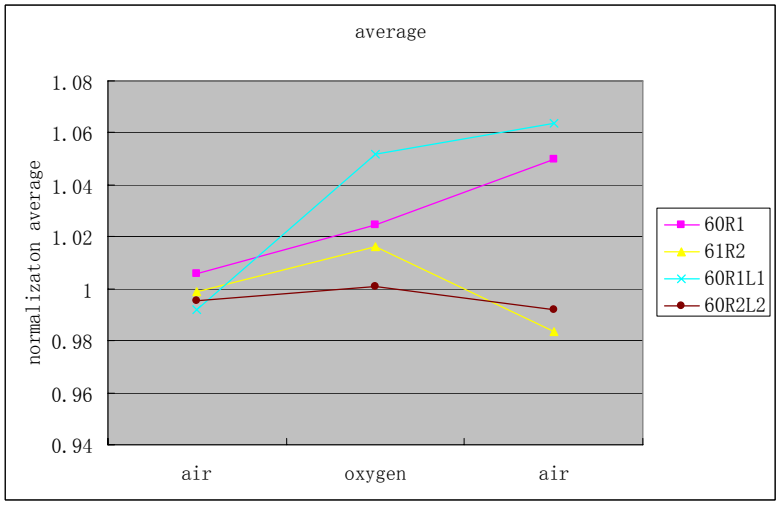


Fig 8. BOLD imaging averaged signal intensity for AT1 tumor

I am continuing analysis particularly to examine original heterogeneity.

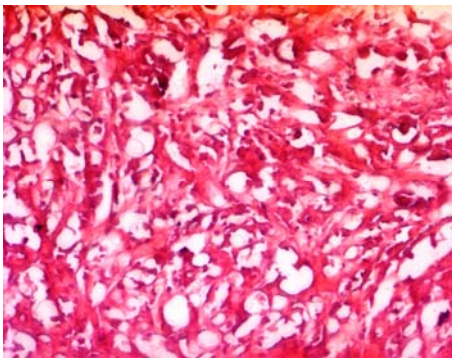
Task 3c. Excise and process tumor tissue for histology

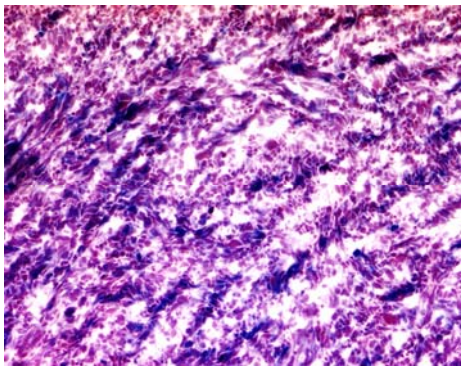
The histology of AT1, H, HI and MAT-Lu rat tumor tissue has been published by our group in several papers. During the three years' training, I got to know the basic concepts of histology

from Dr. LI Liu (a biologist), and also gained hands on some experiences. Here I show the histology of prostate PC3 tumor tissue.

Task 3d. Prepare manuscripts and reports

A manuscript describing measurement of tumor oxygenation with HMDSO as a reporter molecule is published online.





. 9a 9b

Fig 9. Histology of PC3 wild type tumor tissue(9a) and PC3/beta-gal tumor tissue (9b)

Reportable outcomes:

Conference abstracts:

A New Frontier for Proton MRI: Quantitative Tissue Oximetry, Vikram D. Kodibagkar, **Weina Cui**, Ralph P. Mason. Society for molecular imaging, St. Louis, Sep 2004

Quantitative Tissue Oximetry using Proton MR of Hexamethyldisiloxane. Vikram D. Kodibagkar, **Weina Cui**, Metthew. Merritt and Ralph P. Mason, ISMRM, Miami, May 2005

Another related imaging poster based on this grant is accepted by ISMRM, Proton Imaging of Silanes to map Tissue Oxygenation Levels (PISTOL): a new tool for quantitative tissue oximetry. Vikram D. Kodibagkar and Ralph P. Mason, ISMRM, Seattle, May 2006

Paper

Novel 1H NMR approach to quantitative tissue oximetry using hexamethyldisiloxane, Vikram D. Kodibagkar, **Weina Cui**, Metthew. Merritt and Ralph P. Mason, Magnetic Resonance in Medicine, vol55, 743-748, 2006

Grant

Based on the research and results of my project, Dr. Vikram D. Kodibagkar has submitted a pending NIH R21 grant based on these preliminary data on Feb, 2006.

In addition, my grant led me the promotion to instructor in the department of radiology at UTSW. I am continuing prostate cancer research funded by Dr. Mason's new DOD IDEA award (VATUXIMABTM: OPTIMIZING THERAPEUTIC STRATEGIES FOR PROSTATE CANCER BASED ON DYNAMIC MR TUMOR OXIMETRY), as well as pursuing independent projects.

Conclusions:

The above experiments showed that: 1) As a imaging technique, it has been proved that the chemical shift select imaging of HMDSO with water and fat suppression can successfully measure the oxygen tension in rat muscle and tumor; 2) the developed pulse sequence can successfully monitor the rat thigh muscle oxygen dynamics; 3) HMDSO spin-lattice relaxation time (T1) in tumor responds to respiratory intervention (air-oxygen-air); 4) BOLD imaging signal intensity in tumor also responds to respiratory intervention (air-oxygen-air). So, Hexamethyldisiloxane shows promise as a reporter molecule to measure tumor oxygenation by ^{1}H MRS and potentially by MRI. This opens new opportunities for MR tumor oximetry, particularly since HMDSO is used widely in biomedical materials and as an ingredient in consumer products; and HMDSO is reported to have minimal toxicity.

Reference:

(1) Haase, A.; Frahm, J.; Hanicke, W.; Matthaei, D. *Phys Med Biol* **1985**, *30*, 341-4.
(2) Hunjan, S.; Zhao, D.; Constantinescu, A.; Hahn, E. W.; Antich, P. P.; Mason, R. P. *Int. J. Radiat. Oncol. Biol. Phys.* **2001**, *49*, 1097-1108.

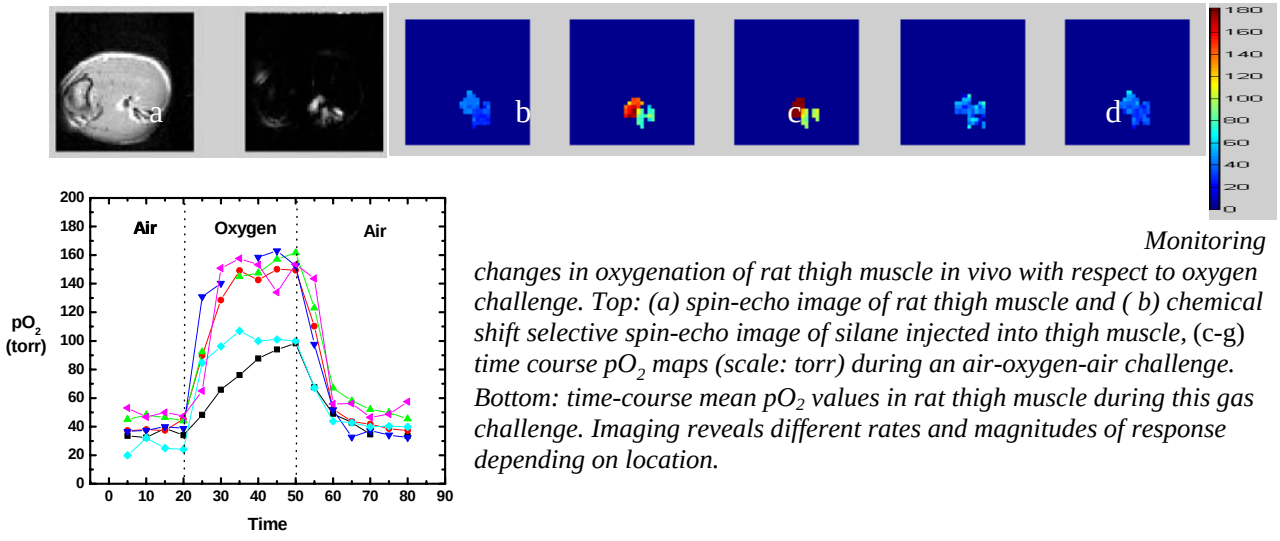
Proton Imaging of Silanes to map Tissue Oxygenation Levels (PISTOL): a new tool for quantitative tissue oximetry

Vikram D. Kodibagkar and Ralph P. Mason
Cancer Imaging Program, Department of Radiology, University of Texas Southwestern Medical Center at Dallas, TX 75390

Introduction: **There is increasing evidence for the importance of tissue oxygenation in development, progression, and response to cancer therapy. Oxygen is required for efficient function by most tissues and hypoxia leads to rapid cellular dysfunction and damage. Thus, the opportunity to measure tissue oxygen tension (pO_2) non-invasively may be significant in understanding mechanisms of tissue function and in clinical prognosis. The potential of hexamethyldisiloxane (HMDSO) as a ^1H based pO_2 reporter molecule (by analogy with fluorinated pO_2 reporters) has been previously studied by ^1H spectroscopy and linear dependence of R_1 of HMDSO on pO_2 (R_1= 0.12+ 0.00173*pO_2 [torr] at 37°C) was observed (1). We have now extended application to present an imaging based method, PISTOL (Proton Imaging of Silanes to map Tissue Oxygenation Levels), and use HMDSO to map tissue oxygenation in rat thigh muscle in response to oxygen challenge**

Materials and Methods: **A spin-echo EPI based pulse sequence was used for imaging and measuring T_1 values using a Varian 4.7 T scanner. The sequence consisted of a) 20 non-selective saturation pulses followed by a delay *tau* for magnetization recovery, b) 3 CHESS pulses for selective saturation of water and fat immediately followed by c) spin-echo EPI detection with a slice selective 90° pulse and a frequency selective 180° pulse. T_1 datasets were obtained using this sequence with the ARDVARC (*A*lternating *R*elaxation *D*elays with *V*ariable *A*cquisitions for *R*eduction of *C*learance effects) protocol (2), by varying *tau* (requiring a total of 3 min. per T1 map). Reference images were also obtained using a spin echo sequence. T_1 and pO_2 maps were computed using homebuilt software based on the Matlab programming language.**

Results and Discussion:



Monitoring changes in oxygenation of rat thigh muscle in vivo with respect to oxygen challenge. Top: (a) spin-echo image of rat thigh muscle and (b) chemical shift selective spin-echo image of silane injected into thigh muscle, (c-g) *time course pO_2 maps (scale: torr) during an air-oxygen-air challenge. Bottom: time-course mean pO_2 values in rat thigh muscle during this gas challenge. Imaging reveals different rates and magnitudes of response depending on location.*

PISTOL successfully monitored the modulation of tissue oxygenation in response to oxygen challenge. The short total acquisition time (3 min per pO_2 measurement) reveals dynamic response to oxygen intervention. This study further validates the use of HMDSO as a pO_2 reporter molecule. We believe that PISTOL has great potential for application in the clinic being a proton MRI approach using techniques, which can be implemented on clinical scanners. Lack of toxicity and commercial availability add to the promise of HMDSO as a pO_2 reporter molecule.

Acknowledgements: This work was supported by Cancer Imaging Program P20 CA086354 and BTRP P41 RR02584

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Quantitative Tissue Oximetry using Proton MR of Hexamethyldisiloxane

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Synopsis

There is increasing evidence for the importance of tissue oxygenation in development, progression, and response to cancer therapy. Thus, the opportunity to measure tissue oxygen tension (pO_2) non-invasively may be significant in understanding mechanisms of tissue function and in clinical prognosis. The linear dependence of the spin lattice relaxation rate, R1, of the ^{19}F NMR resonances of fluorocarbons on pO_2 is well known and has been studied extensively. We recently presented hexamethyldisiloxane (HMDSO) as a potential analogous ^{1}H NMR pO_2 reporter molecule . HMDSO has a single proton NMR resonance, which is ideal for imaging. 1H MRI of reporter molecules is subject to potential interference from the large water and fat resonances, but the chemical shift of –5 ppm relative to water allows chemical shift selective imaging. We now demonstrate application with bulk pO_2 measurements and pO_2 maps obtained *in vivo* in rat thighs and tumors following a direct intra tissue injection of 100 µl HMDSO. Dynamic changes in pO_2 were assessed with respect to respiratory challenge. Given the minimal toxicity and ready availability of HMDSO, we believe it has great potential for ultimate application as a pO_2 reporter molecule in the clinic.

Introduction: **There is increasing evidence for the importance of tissue oxygenation in development, progression, and response to cancer therapy. Oxygen is required for efficient function by most tissues and hypoxia leads to rapid cellular dysfunction and damage. In addition, hypoxic tumor cells are refractory to radiotherapy. Thus, the opportunity to measure tissue oxygen tension (pO_2) non-invasively may be significant in understanding mechanisms of tissue function and in clinical prognosis. The linear dependence of R_1 of fluorocarbon ^{19}F resonances on pO_2 is well known and has been studied extensively[1]. We have previously studied the potential of HMDSO as a ^{1}H based pO_2 reporter molecule and found the linear dependence of R_1 of HMDSO on pO_2 (R_1= 0.12+ 0.00173*pO_2[torr] at 37°C). Here, we study the modulation of tissue oxygenation in response to oxygen challenge in order to further validate the use of HMDSO as a pO_2 reporter molecule.**

Materials and Methods: **A spin-echo EPI based pulse sequence was used for imaging and measuring T_1 values using a Varian 4.7 T scanner. The sequence consisted of a) 20 non-selective saturation pulses followed by a delay *tau* for magnetization recovery, b) 3 CHESS pulses for selective saturation of water and fat immediately followed by c) spin-echo EPI detection with a slice selective 90° pulse and a frequency selective 180° pulse. T_1 maps were obtained using this sequence with the ARDVARC (*A*lternating *R*elaxation *D*elays with *V*ariable *A*cquisitions for *R*eduction of *C*learance effects) protocol[2], by varying *tau* (3 and half min. per T1 measurement). For comparison, reference images were obtained using a spin echo sequence. T_1 and pO_2 maps were computed using homebuilt software based on the Matlab programming language.**

Results and Discussion:

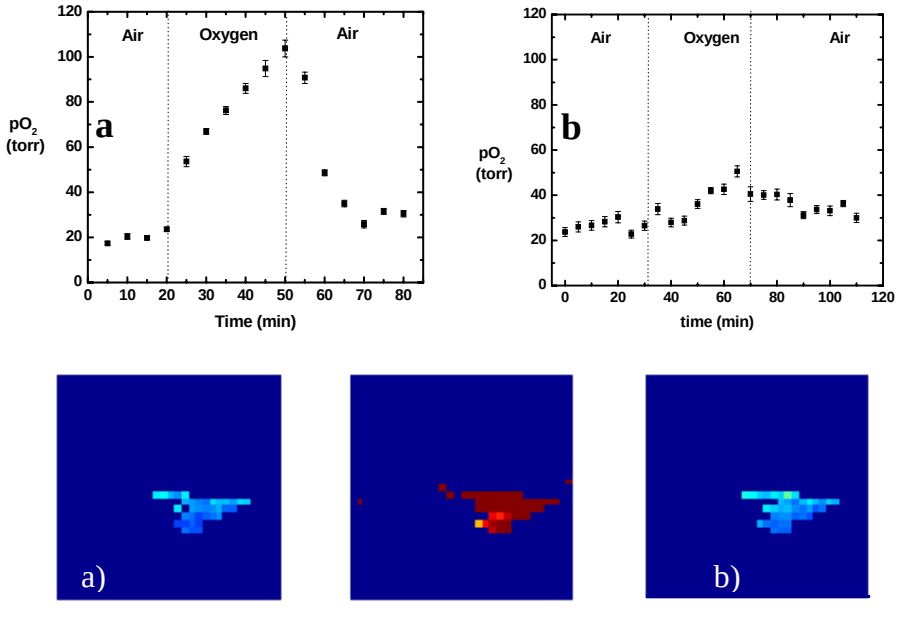

Fig1: *Monitoring modulation of tissue oxygenation by bulk spectroscopy in* ***a)*** *healthy rat thigh and* ***b)*** *rat Dunning prostate AT1 tumor (volume: 5.8 cc)*

Fig2: *Monitoring modulation of tissue oxygenation in response to oxygen challenge in healthy rat thigh* ***a)*** *Air breathing,* ***b)*** *after breathing oxygen for*

*30 min and **c)** 30 min after reverting to air*

Modulation of tissue oxygenation in response to oxygen challenge was successfully monitored by bulk spectroscopy and imaging. The short total acquisition time reveals dynamic response to therapeutic interventions. Minimal toxicity and wide availability add to the promise of HMDSO as a pO_2 reporter molecule. We believe it has great potential for application in the clinic especially as all the techniques used can be implemented on clinical scanners.

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